



Authorizations and Permits for Protected Species (APPS)

File #: 19571

Title: Recovery Tool for the Endangered Black Abalone

Applicant Information

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Project Information

File Number: 19571
Application Status: Application Complete - Issued
Project Title: Recovery Tool for the Endangered Black Abalone
Project Status: New
Previous Federal or State Permit:
Permit Requested: • ESA Section 10(a)(1)(A) permit (Pacific fish/invertebrate research)
Where will activities occur? California (including offshore waters)
State department of fish and game/wildlife: N/A
Research Timeframe: Start: 08/12/2016 End: 12/31/2020

Sampling Season/Project Duration: The project duration is expected to be five years, beginning as soon as permits are issued. The project will lead to the development of methods for reliable production of high quality juveniles that can be used for future outplanting activities in support of recovery efforts. The SWFSC is equipped with systems where temperature and photoperiod can be manipulated to mimic natural conditions. Therefore, sampling of animals and experiments will be conducted on a year round basis. Conditioning abalone will be a daily occurrence where food, temperature, and light exposure will be monitored and optimized. Checking gonadal development and spawning experiments will happen quarterly or no more than 4 times per year. If spawning attempts are successful, veliger stage animals will be subsampled daily for survival estimates. Growth and survival data will be recorded on a monthly basis.

Abstract: The black abalone, *Haliotis cracherodii*, is listed as endangered under the ESA. SWFSC received 10 black abalone from SSC PACIFIC on 12/19/2014. We plan to investigate conditioning cues and appropriate spawning methods for this species. The main purpose of this research is to develop successful techniques for consistent production of high quality juvenile black abalone to support future research and outplanting efforts. The research will also support development of management strategies necessary for successful recovery of this species and possibly assist natural resource managers in selecting the location and size of marine protected areas (MPAs) designed to protect black abalone. Prior efforts to spawn and produce black abalone spat have been largely unsuccessful. The proposed work seeks to better condition black abalone for successful spawning and improve fertilization success, settlement, and recruitment.

We also seek permission to work with other institutions that have captive black abalone populations (e.g., UCSB, CDFW, and the Monterey Bay Aquarium). Additionally, we are seeking permission to house other black abalone that may become available through confiscations due to law enforcement cases, emergency response activities, or from projects covered under ESA-Section 7 consultations. We also seek permission to work with researchers in Mexico, to exchange live animals, samples, and specimens for research. By collaborating with these institutions we can increase the number of spawning abalone broodstock and likely resulting juveniles to maximize success.

The manner of take spans across several categories: captive research, enhancement and public display; including breeding, lab experiments, tissue sampling, and observation. The main tissue sampling would be either to preserve any mortalities or moribund animals, and also collection of samples for genetic purposes. All animals will be housed at the SWFSC Aquarium Culture Facility.

Project Description

Purpose: The black abalone, *Haliotis cracherodii*, is listed as endangered under the ESA. It is critical to understand the various reproductive factors that increase larval survival and settlement. This will ultimately lead to the development of methods for reliable production of high quality juveniles that can be used for future outplanting activities in support of recovery efforts. Prior efforts to spawn and produce black abalone spat have met with negative results. Through appropriate conditioning of black abalone (manipulating diet, temperature, photoperiod regimes, and possibly water depth to mimic tidal fluctuations), we hope to increase spawning success in captivity, which will facilitate improved fertilization, larval rearing, and juvenile production research.

Within California, several species of commercially valuable abalone are routinely reared in hatcheries using induced spawning techniques. Successful spawning of California abalone species (*H. rufescens*, *H. fulgens*, *H. corrugata*) and Indo Pacific species (*H. discus hannai*, *H. discus*, *H. diversicolor*, *H. supertexta*, *H. laevigata*, *H. rubra*; *H. assisina*) in captivity has been ongoing in some cases since the 1960s. Necessary to the rearing and production of abalone in a hatchery setting is the capacity to condition them for induced spawning. This is accomplished by providing adequate levels of appropriate kelp and other macroalgae, suitable water culture temperatures for each species to accelerate the development of gonads in the male and female broodstock, and the precise timing of the release of gametes (eggs and sperm) in a controlled manner to maximize fertilization

rates.

The following section provides a description of proposed research activities. Once captive breeding techniques have been developed, they can be used to grow larger numbers of high quality juvenile abalone for future outplanting purposes. It is anticipated that broodstock conditioning will begin as early as fall 2015, followed by induced spawning attempts by winter/spring 2016.

Description: Description of Proposed Research Activities for Black Abalone at SWFSC:

1. Conditioning black abalone for spawning

Although the aquaculture of many abalone species is a large industry, only a few attempts have been made to spawn black abalone and most have been unsuccessful. The spawning conditions that will be investigated include diet, culture temperature, and photoperiod manipulation. We propose to condition the abalone using different proportions of brown and red macroalgae harvested in local waters, which will be provided to the abalone twice a week. Conditioning will be conducted in dedicated broodstock systems with the abalone attached to clear plastic plates. These plates will allow for a rough estimate of the gonad index to be assessed once every two weeks without disturbing the animals by detaching them from their substrate. If gonad index cannot be properly assessed through the clear plate, abalone will be removed from the plates with a broad spatula according to standard practice (this will not occur more than once a month). All other handling of the abalone will be minimized to prevent undue stress to the animals. Each animal will only be examined out of the water for as short a time period as needed to determine sex, gonad index, weight, and size (typically less than 5 minutes), no more than once per month.

Two weeks of intense feeding are usually required to hasten the development of male and female gonads at the appropriate time in Southern California where temperatures typically range from 14-18°C. The appropriate temperature for conditioning the green abalone lies in the range of 18-22°C. The black abalone lives in a temperature regime of 7-24°C, but appears capable of spawning in the spring and summer months from April through September in Monterey Bay (Webber and Giese, 1969), a time of increasing water temperatures. Other studies found evidence that the black abalone spawns in Southern California waters in late summer and early fall (temperatures range from 19-22°C) (Leighton, 1959; Leighton and Boolootian, 1963). This background information will be used to attempt to initiate increases in gonadal indices in our temperature controlled broodstock tanks.

Gonadal indices are identified by stages 1-3 (Leighton, 2000). Stage 1: the gonad mass is below the shell margin, stage 2: the gonad mass has enlarged to the extent that it is level with the shell margin, and stage 3: the gonadal mass extends above the rear margin of the shell. This conditioning index is used for both males and females. Lack of development of the gonads to a spawnable condition is probably due to incorrect temperatures present and equally important, the nutritional quality of the diet. The brown kelps, *Macrocystis pyrifera* and *Egregia laevigata* are most commonly encountered in the middle and high intertidal coastal zones as drift. These kelps have been shown to elicit differences in growth rates of red and green abalone at various temperatures and seasonally (Lapota, 1982; 2000). Hatchery procedures for conditioning broodstock have been widely discussed and published for other green species (Hahn, 1989; Heasman and Savva, 2007) and serve as the basis for our proposed conditioning of black abalone. We plan to simultaneously test various algal feed protocols as follows:

- 1). 2-3 abalone will be fed ad libitum on a mixture of brown macroalgae for 1 month at each temperature: 17°C, 19°C, and at 21°C. Total time for conditioning is 3 months.
- 2). 2-3 abalone will be fed ad libitum on a select red macroalgae for 1 month at each temperature: 17°C, 19°C, and at 21°C. Total time for conditioning is 3 months.
- 3). 2-3 abalone will be fed ad libitum on an artificial diet for 1 month at each temperature: 17°C, 19°C, and at 21°C. Total time for conditioning is 3 months.

At the end of each three month diet/temperature treatment period, we will attempt to spawn the abalone (see #2, "Cues for spawning" below), as long as gonad condition requirements are met. If needed, we will extend the time for gonad conditioning.

2. Cues for spawning: Thermal shock and hydrogen peroxide protocols

Standard and modified methods used to induce spawning in other species of abalone will be investigated on conditioned black abalone. Males and females will be induced to spawn in separate tanks using a solution of Tris-buffered seawater and hydrogen peroxide, with a final concentration of 6% H₂O₂. Following 3 hours of exposure, the seawater containing hydrogen peroxide will be poured off and replaced with filtered seawater. Following 30 minutes of spawning, eggs and sperm will be collected and mixed to settle. Fertilized eggs will then be washed in copious amounts of filtered seawater to remove excess sperm in order to minimize chances of polyspermy. Eggs will then be transferred to hatch-out tubs. An egg count will be made by subsampling the eggs liberated following fertilization. Within 18 hours following fertilization, trochophores will hatch from the eggs, rise to the surface of the container and swarm. Emergent trochophores will be subsampled and examined microscopically to determine the number actively swimming.

Thermal shock techniques to induce spawning, if needed, will be closely monitored and controlled. Suggested thermal treatments are short in duration (0.5 hr) and will increase water temperatures by 3-4°C, but still stay within the thermal tolerance range for this species (7-24°C). Additionally, water depth may be manipulated or tidal sequences simulated by changing the water level in the tanks (from a few inches to 12 - 24 inches), to determine possible thermal cues based on tidal regimes. We will closely monitor the animals for signs of stress during spawning activities.

We anticipate using two adult males and 3-4 adult females for each spawning experiment, depending on the sex ratio of the captive animals. We hope to have 3-4 spawning experiments within the first year of the permit being granted.

3. Settlement of veligers: GABA induction/substrate choice

We will evaluate which larval rearing method supports greater larval survival. Some treatments may have filtered seawater trickling into the tanks for comparison to static renewal treatments (no flowing water; daily partial water changes). Repeated spawnings should reveal ideal settling conditions and increased veliger vigor. At approximately 48 hours of age, veligers will be subsampled microscopically. The late stage veligers are attracted to settle on films of diatoms, crustose algae, or by the addition of Gamma Amino Butyric Acid (GABA), a neurotransmitter. Several concentrations of GABA will be tested to induce the veligers to settle. A range of antibiotics and probiotics may also be tested during the settlement process to improve survival rates and maintain healthy microbial conditions. Oxytetracycline and Gentamycin have been used to settle red and green abalone at other facilities with encouraging results. Other approaches to induce settlement may include making a slurry of coralline algae and rocks covered with coralline algae.

Settled veligers will be housed in separate larval rearing vessels apart from the adult abalone. Various substrates will be used to settle the veligers. Planned experimental treatments may include: using a range of diatoms species (composite), single species of diatoms, antibiotic treatments, crustose coralline algae, coralline slurry, artificial seawater, and mild aeration. Replicates of each treatment will be set up during the settlement phase for statistical analyses. Seawater will be partially changed on a daily basis. As the juveniles increase in size, diets will initially consist of benthic diatoms. Settled juvenile abalone will be allowed to graze on the diatom-film-coated tank walls until large enough to be transferred to larger nursery tanks for weaning onto the larger brown kelps collected on a regular basis. Once the juveniles reach a size of 6 mm, red and brown macroalgae will be added.

Settled veligers (veligers exhibiting pedal locomotion) will also be subsampled to estimate survival. Subsequent microscopic counts on the settled juveniles will be conducted monthly.

Throughout the larval development stage, we will subsample the larvae to determine survival rates. Most larvae will be returned to rearing systems but a small number (10 individuals) will be preserved to document development at each of the following stages: egg, trochophore larvae, veliger larvae, and settled larvae. After larval settlement, we will subsample settled veligers (veligers exhibiting pedal locomotion) to estimate survival. We will subsequently conduct microscopic counts on settled juveniles on a monthly basis to determine settlement rates, until the animals are large enough to be seen without a microscope. To document development of post-settlement juveniles, we will collect and preserve 10 individuals per week until 3 months of age. From 3-6 months of age, we will collect and preserve 5 individuals per month. Thereafter, we will document development using non-lethal methods (measuring lengths and weights of individuals)

4. Growth and survival

Growth will be monitored in both adults and progeny. Adult growth will be assessed by both shell size and observation of new growth on the shell margin. Juveniles will be measured for shell growth and weight once they reach 12 mm in size. Previous studies on other abalone species have shown that growth in juveniles is dependent on diet and water temperature. Different thermal treatments will be used to determine optimal growout temperatures for black abalone juveniles. The effects of different temperatures on growth rate should be obvious within several months. Fastest growth rates in green abalone have been observed at a constant temperature of 21°C (Lapota 1982; Lapota et al., 2000). Others have observed maximum growth rates of juveniles at higher water temperatures (Heasman et al. 2006; Williams et al., 2008).

We will hold captive-bred juveniles at three different temperatures (17, 19, and 21°C) for three to six months. About 50 – 100 juveniles with an initial size of about 12 mm shell length will be used for each treatment. Growth and survival data will be recorded on a monthly basis.

If the thermal treatments do not show a difference in growth with temperature, then we may evaluate how diet affects growth, using replicated experiments with different types of macroalgae known to be eaten by abalone and artificial manufactured feed. We will measure monthly growth and survival.

5. Collaboration with other facilities

In the hatchery setting, mortality is high among the different stages of development, with an average of 1% survival to the settled-juvenile stage. For example, if we collect 50,000 fertilized eggs from a successful black abalone spawn, 40,000 trochophores may hatch; 20,000 veligers may develop, and only 4,000 veligers may settle. Attrition continues through metamorphosis, which could result in 400 juveniles. As we only have eight adult black abalone as potential broodstock we are somewhat limited in our ability to successfully conduct all the research activities proposed above. Therefore, the ability to work with collaborating institutions that currently or may hold black abalone, such as UCSB, University of California Davis – Bodega Marine Lab (UCD BML), the Monterey Bay Aquarium, and the Center for Scientific Research and Higher Education of Ensenada, Baja California (CICESE), to obtain more animals to use as broodstock at the SWFSC would greatly enhance our efforts. At this time, spawning events and research activities involving live black abalone will only occur at the SWFSC, not at other facilities. However, we may request to conduct these activities at other facilities in the future.

We seek permission to transfer black abalone from other captive facilities to the SWFSC. Additionally, we seek permission to obtain coverage for black abalone that may become available from law enforcement confiscations, emergency response activities, and projects covered under ESA-Section 7 consultations. We anticipate obtaining up to 16 additional black abalone from other facilities and up to 130 animals black abalone adults and/or juveniles from confiscations from law enforcement, projects covered under ESA Section 7 consultations, and/or emergency response activities.

For transfers from UCSB (which currently has about 20 pre-listed black abalone), we will coordinate with Scott Simon in Santa Barbara once we receive permission to transfer. Other facilities in the U.S. that currently hold pre-listed black abalone include the UC Davis – Bodega Marine Lab (about 20 animals), and Monterey Bay Aquarium (number of animals unknown). For transfers from these facilities, we will request permission and coordinate with the appropriate point of contact at the facility. Laboratories at CICESE in Ensenada, Baja California, may also hold adult and/or juvenile black abalone that may be transferred to the SWFSC. For transfers from CICESE, we will request permission and coordinate with Dr. Fabiola LaFarga de la Cruz.

For law enforcement confiscations, we will coordinate with John Potter of CDFW in San Diego. We anticipate the types of cases in which black abalone would become available would be from poaching. For projects covered under ESA Section 7 consultations, we would be able to receive black abalone should they need to be removed from the wild due to actions covered under ESA Section 7 consultations and/or emergency response activities (Oil response). The take of black abalone from the wild in these instances would be analyzed and covered by the appropriate ESA process for the action: this permit would cover the SWFSC's receipt of and research on those animals once brought into captivity.

Dr. Fabiola LaFarga's lab at CICESE may also attempt to captively breed black abalone for research purposes. The ability to exchange broodstock with Dr. LaFarga's lab would allow collaboration between our labs and increase the potential for developing reliable captive breeding methods. In addition, the ability to exchange captive-bred gametes and progeny (embryos, larvae, juveniles) with Dr. LaFarga's lab would also enhance our efforts to develop culture methods and learn about the species' life history and biology, particularly if one lab is more successful than the other at captively breeding the animals. Therefore, we also propose to receive and/or transfer captive-bred gametes (cryopreserved) and/or progeny (embryos, larvae, juveniles) with Dr. LaFarga's lab. We describe the methods of transport for these early life stages in the "Transport Information" section below. Given the high fecundity of abalone, these transfers may involve millions of gametes, embryos, and/or larvae, and thousands of juveniles.

Other Information:

SWFSC Aquarium Culture Facility: The SWFSC Aquarium is an established 80-100 gpm seawater flow-through facility located in La Jolla, CA. Unfiltered seawater is pumped from the Scripps Institution of Oceanography pier, through a series of sand filters and up to the main laboratory building, where it is filtered through a series of sand filters, cartridge filters, UV sterilization units, ozone sterilization, and activated carbon filtration. It is then delivered to the aquarium facility and distributed amongst 10 sets of temperature mixing stations, with varying system volumes and sizes. Temperature is controlled at each mixing station by a centralized computer and remotely monitored by two redundant systems. Temperatures can be manipulated in each of the mixing stations at ranges from 5 to 30 °C. Within the aquarium, there are 4 rooms dedicated to abalone culture; 2 rooms for broodstock abalone, a wet lab for larval rearing, and a separate dry lab for microscopy and lab analyses. On larval rearing systems, there is additional 1-5 micron filtered water and UV sterilization. All adult abalone are housed in separate holding systems (pink, green, and black), and are not transferred into broodstock holding rooms until treated and testing negative for Withering Foot Syndrome (WS). All tanks are aerated continuously. Outgoing water is discharged through facility discharge drains. The black abalone are currently housed in a 4' x 2' x 1' shallow 30 gallon tank at 15 °C and separate from our pink and green abalone populations.

Disease Control: The Aquarium facility is inspected bi-annually for sabellid worm infestations and WS by the California Department of Fish and Wildlife. Cultured pink abalone at the facility have been sabellid free and tested negative for WS since 2012. The black abalone have tested positive for WS upon arrival from SSC Pacific and will be treated with Oxytetracycline dihydrate per established disease management protocols from CDFW. Strict biosecurity practices will be in place at all times.

Daily or alternate day passive tank transfers of pre-settled larvae will occur, in addition to partial water changes on a daily basis after larval settlement. This will allow for control of bacterial infestations and maintenance of necessary beneficial microbial communities on early and late stage veligers and less frequently on settled juveniles. Oxytetracycline and Gentamycin may also be used in addition to these methods.

Stress Management: To control stress to the black abalone during induced spawning attempts, abalone will not be exposed to elevated hydrogen peroxide for longer than the published guidelines (2.5 - 3 hours of exposure). All treated abalone will be washed in fresh seawater to remove residual levels to permit spawning. Thermal shock techniques, if needed, will be closely monitored and controlled. Suggested thermal treatments are short in duration (0.5 h) and will increase water temperatures by 3-4°C, but still stay within the tolerance range for this species (7-24°C).

Frequency of Spawning: Abalone will only be induced to spawn should the gonad conditioning criteria be met. It may require longer periods of time to condition these animals than previously reported. No more than four attempts to induce spawning will occur within a one year period. The number of individuals used will depend on the sex ratio of the captive animals. Buffered seawater containing hydrogen peroxide is the industry standard for induced spawning; however, the thermal shock method may be used if the former method fails to trigger spawning. Released eggs, swimming trochophores, and developing veligers will be subsampled to assess initial numbers of each stage. Settled veligers will also be enumerated microscopically. With this sequence, we will be able to determine the survival rate through all stages of development (% survival).

Disposition of Tissues: In the event of mortalities, tissues will be preserved for subsequent analysis and per the protocols developed for the White Abalone Recovery Program. These tissues will be maintained at SWFSC except for those sent to CDFW for post-mortem examination and to other labs (e.g., UW, NWFSC, CICESE) for analysis and/or research (e.g., genetic studies). Samples of larval and juvenile abalone will also be collected and preserved to document development. Tissue samples will also be taken for genetic purposes. These can be taken from fresh dead animals, or small clips of epipodial tissue from live animals. Tissue samples may be maintained at the SWFSC as well as transferred to co-investigators at other facilities (UW, NWFSC, CDFW, CICESE) for analysis.

Supplemental Information

Status of Species: In the 2009 black abalone status review, VanBlaricom et al. (2009) stated that black abalone are unable to recover their populations naturally. "The spread of the disease now extends over almost the entire range of the species. Withering Syndrome does not appear to show any signs of relenting in its progression along the coast in either direction. The strong correlation between adult abundance and recruitment suggests that larvae do not disperse very far from their point of origin, thus depleted abalone populations are unlikely to be restored by recruitment from distant populations. If no action is taken, it is estimated that *H. cracherodii* will decline by at least 80% over a period of three generations (from approximately 1975 to 2015) (Smith et al. 2003)." On recent surveys conducted on San Clemente Island (SCI), approximately 30 black abalone were found. On San Nicholas Island (SNI), low numbers of black abalone were located. VanBlaricom et al. (2009) reported densities of less than 0.5 abalone/square meter - too low to sustain and increase abalone populations and concluded that the black abalone will likely face extinction in less than 30 years without substantial recovery efforts.

Methods: The methods for conditioning broodstock (temperature, feeding, photoperiod manipulations), settlement of veligers, and growth and survival are described below. These methods closely follow the guidelines and protocols developed for the White Abalone Restoration Program for spawning, rearing, tissue sampling and necropsy procedures. See attached.....

1. Conditioning black abalone for spawning

Although the aquaculture of many abalone species is a large industry, only a few unsuccessful attempts have been made to spawn black abalone. The spawning conditions that will be investigated include diet, culture temperature, and photoperiod manipulation. We propose to condition the abalone using different proportions of

brown and red macroalgae harvested in local waters, which will be provided to the abalone twice a week. Conditioning will be conducted in dedicated broodstock systems with the abalone attached to clear plastic plates. These plates will allow for a rough estimate of the gonad index to be assessed once every two weeks without disturbing the animals by detaching them from their substrate. If gonad index cannot be properly assessed through the clear plate, abalone will be removed from the plates with a broad spatula according to standard practice (this will not occur more than once a month). All other handling of the abalone will be minimized to prevent undue stress to the animals. Each animal will only be examined out of the water for as short a time period as needed to determine sex, gonad index, weight, and size (typically less than 5 minutes).

Two weeks of intense feeding are usually required to hasten the development of male and female gonads at the appropriate time in Southern California where temperatures typically range from 14-18°C. The appropriate temperature for conditioning the green abalone lies in the range of 18-22°C. The black abalone lives in a temperature regime of 7-24°C, but appears capable of spawning in the spring and summer months from April through September in Monterey Bay (Webber and Giese, 1969), a time of increasing water temperatures. Other studies found evidence that the black abalone spawns in Southern California waters in late summer and early fall (temperatures range from 19-22°C) (Leighton, 1959; Leighton and Boolootian, 1963). This background information will be used to attempt to initiate increases in gonadal indices in our temperature controlled broodstock tanks.

Gonadal indices are identified by stages 1-3 (Leighton, 2000). Stage 1: the gonad mass is below the shell margin, stage 2: the gonad mass has enlarged to the extent that it is level with the shell margin, and stage 3: the gonadal mass extends above the rear margin of the shell. This conditioning index is used for both males and females. Lack of development of the gonads to a spawnable condition is probably due to incorrect temperatures present and equally important, the nutritional quality of the diet. The brown kelps, *Macrocystis pyrifera* and *Egregia laevigata* are most commonly encountered in the middle and high intertidal coastal zones as drift. These kelps have been shown to elicit differences in growth rates of red and green abalone at various temperatures and seasonally (Lapota, 1982; 2000). Hatchery procedures for conditioning broodstock have been widely discussed and published for other species (Hahn, 1989; Heasman and Savva, 2007) and serve as the basis for our proposed conditioning of black abalone. We plan to simultaneously test various algal feed protocols as follows:

- 1). 2-3 abalone will be fed ad libitum on a mixture of brown macroalgae for 1 month at each temperature: 17°C, 19°C, and at 21°C. Total time for conditioning is 3 months.
- 2). 2-3 abalone will be fed ad libitum on a select red macroalgae for 1 month at each temperature: 17°C, 19°C, and at 21°C. Total time for conditioning is 3 months.
- 3). 2-3 abalone will be fed ad libitum on an artificial diet for 1 month at each temperature: 17°C, 19°C, and at 21°C. Total time for conditioning is 3 months.

At the end of each three month diet/temperature treatment period, we will attempt to spawn the abalone (see "Cues for spawning" below), as long as gonad condition requirements are met. If needed, we will extend the time for gonad conditioning.

2. Cues for spawning: Thermal shock and hydrogen peroxide protocols

Standard and modified methods used to induce spawning in other species of abalone will be investigated on conditioned black abalone. Males and females will be induced to spawn in separate tanks using a solution of Tris-buffered seawater and hydrogen peroxide, with a final concentration of 6% H₂O₂. Following 3 hours of exposure, the seawater containing hydrogen peroxide will be poured off and replaced with filtered seawater. Following 30 minutes of spawning, eggs and sperm will be collected and mixed to settle. Fertilized eggs will then be washed in copious amounts of filtered seawater to remove excess sperm in order to minimize chances of

polyspermy. Eggs will then be transferred to hatch-out tubs. An egg count will be made by subsampling the eggs liberated following fertilization. Within 18 hours following fertilization, trochophores will hatch from the eggs, rise to the surface of the container and swarm. Emergent trochophores will be subsampled and examined microscopically to determine the number actively swimming.

Thermal shock techniques to induce spawning, if needed, will be closely monitored and controlled. Suggested thermal treatments are short in duration (0.5 hr) and will increase water temperatures by 3-4°C, but still stay within the thermal tolerance range for this species (7-24°C). Additionally, water depth may be manipulated or tidal sequences simulated to determine possible thermal cues based on tidal regimes. Water depth manipulations and tidal sequence simulations would involve changing the water level in the tanks, ranging from a few inches to 12 to 24 inches. We would closely monitor the animals for signs of stress during spawning activities.

It is anticipated that we will use two adult males and 3-4 adult females for each spawning experiment, depending on the sex ratio of the captive animals. We hope to have 3-4 spawning experiments within the first year of the permit being granted.

3. Settlement of veligers: GABA induction/substrate choice

We will evaluate larval rearing methods to determine which methods support greater larval survival. Some treatments may have filtered seawater trickling into the tanks for comparison to static renewal treatments (no flowing water; daily partial water changes). At approximately 48 hours of age, veligers will be subsampled microscopically. Most larvae will be returned to rearing systems but a small number (10 individuals) will be preserved to document development at each of the following stages: egg, trochophore larvae, veliger larvae, and settled larvae. The late stage veligers are attracted to settle on films of diatoms, crustose algae, or by the addition of Gamma Amino Butyric Acid (GABA), a neurotransmitter. Several concentrations of GABA will be tested to induce the veligers to settle. A range of antibiotics and probiotics may also be tested during the settlement process to improve survival rates and maintain healthy microbial conditions. Oxytetracycline and Gentamycin have been used to settle red and green abalone at other facilities with encouraging results. Other approaches to induce settlement may include making a slurry of coralline algae and rocks covered with coralline algae.

Settled veligers will be housed in separate larval rearing vessels apart from the adult abalone. Various substrates will be used to settle the veligers. Planned experimental treatments may include: using a range of diatoms species (composite), single species of diatoms, antibiotic treatments, crustose coralline algae, coralline slurry, artificial seawater, and mild aeration. Replicates of each treatment will be set up during the settlement phase for statistical analyses. Seawater will be partially changed on a daily basis. Repeated spawnings should reveal ideal settling conditions and increased veliger vigor. As the juveniles increase in size, diets will initially consist of benthic diatoms. Settled juvenile abalone will be allowed to graze on the diatom-film-coated tank walls until large enough (about 6mm in size) to be transferred to larger nursery tanks for weaning onto the larger red and brown macroalgae collected on a regular basis.

Settled veligers (veligers exhibiting pedal locomotion) will also be subsampled to estimate survival. To document development of post-settlement juveniles, researchers will collect and preserve 10 individuals per week until 3 months of age. From 3-6 months of age, researchers will collect and preserve 5 individuals per month. Thereafter, development will be documented using non-lethal methods (measuring lengths and weights of individuals).

4. Growth and survival

Growth will be monitored in both adults and progeny. Adult growth will be assessed by both shell size and observation of new growth on the shell margin. Juveniles

will be measured for shell growth and weight once they reach 12 mm in size. Previous studies on other abalone species have shown that growth in juveniles is dependent on diet and water temperature. Different thermal treatments will be used to determine optimal growout temperatures for black abalone juveniles. The effects of different temperatures on growth rate should be obvious within several months. Fastest growth rates in green abalone have been observed at a constant temperature of 21°C (Lapota 1982; Lapota et al., 2000). Others have observed maximum growth rates of juveniles at higher water temperatures (Heasman et al. 2006; Williams et al., 2008).

We will hold captive-bred juveniles at three different temperatures (17, 19, and 21°C) for three to six months. About 50 – 100 juveniles with an initial size of about 12 mm shell length will be used for each treatment. Growth and survival data will be recorded on a monthly basis.

If the thermal treatments do not show a difference in growth with temperature, then we may evaluate how diet affects growth, using replicated experiments with different types of macroalgae known to be eaten by abalone and artificial manufactured feed. We will measure monthly growth and survival.

5. Captive maintenance, grow-out, and sampling

We will maintain and grow-out the broodstock and captive-bred progeny using established holding protocols for abalone, adjusted to optimize conditions for black abalone based on the studies described above. On a regular basis, we will remove animals from the substrate to measure their shell length, weight, and gonad condition. We will remove animals from the substrate by hand or by using kelp, an abalone iron, a plastic spatula, or another flat instrument with a thin profile and blunt edge. We may apply tags to individuals for identification, using shell banding, external tags attached to the shell (e.g., numbered bee tags or vinyl shellfish tags attached with marine epoxy or superglue), or external tags embedded in the shell (Passive integrated transponder or PIT tags; see Hale et al. 2012 or Richards and Whitaker 2012). We will also collect tissue samples (epipodial clippings) from juvenile and adult abalone for genetic analysis, using well-established, non-lethal methods (Hamm and Burton 2000). We will use tweezers to grasp the epipodial tentacles on the sides or posterior of the animal and cut the tentacle no closer than 1-2 millimeters from its base.

We will regularly monitor the health of the animals and conduct health treatments when needed. Specific health monitoring and treatments include:

(a) Withering syndrome monitoring and treatment: Withering syndrome causes the animal's foot muscle to wither and eventually kills the animal. The disease is caused by a pathogen (WS-RLO) transmitted fecal-orally. To assess whether the animals are infected with the WS-RLO, we will regularly collect fecal samples for analysis by CDFW-BML. If needed, we will treat infected animals with oxytetracycline (OTC), using the protocol established by Jim Moore (2015; CDFW-BML). This involves immersing the abalone in an OTC bath solution for 24 hours at a time, for eight baths in total.

(b) Shell waxing to remove shell-boring organisms: Shell-boring organisms can infest and weaken the abalone's shell, leading to shell damage and potentially to death. If needed, we will apply a wax treatment (Moore and Marshman 2015) to remove *Polydora* or heavy infestations of shell-boring organisms. Wax treatment involves removing the abalone from the substrate, scrubbing the shell surface with a brush, and coating the shell surface with a wax mixture (beeswax and coconut oil), taking care not to cover the respiratory pores. The animals would not be out of the water for more than 10 minutes.

(c) Sabellid worm inspections: Parasitic sabellid polychaete worms can infest the growing edge of shells and cause shell deformity, slow growth, and brittleness. The SWFSC lab has already been certified as sabellid-free and undergoes regular inspections by CDFW, about once per year. Inspections involve removing the abalone from the holding tanks and visually inspecting individuals for the presence of sabellid worms. Depending on the number of animals at the facility, all or a subset of

animals at each facility may be examined. Animals are out of the water for no more than 30 minutes.

We will intentionally kill obviously dying black abalone for necropsy, by freezing whole animals, or dissecting the relevant tissues (gut and foot muscle) and either freezing the tissues or fixing them in formalin before placing in ethanol. This will preserve the tissues for analysis. Obviously dying individuals are those that show the following symptoms: extreme lethargy, withered and discolored foot muscle, and/or inability to hold onto the substrate. Specimens and/or tissues/parts will be analyzed at the SWFSC or at labs listed as co-investigators on the permit, including the University of Washington (UW), NMFS Northwest Fisheries Science Center (NWFSC), University of California Davis – Bodega Marine Lab (UCD BML), or the Center for Scientific Research and Higher Education of Ensenada, Baja California (CICESE).

Disease Control: The Aquarium facility is inspected bi-annually for sabellid worm infestations and WS by the California Department of Fish and Wildlife. Cultured pink abalone at the facility have been sabellid free and tested negative for WS since 2012. The black abalone have tested positive for WS upon arrival from SSC Pacific and will be treated with Oxytetracycline dihydrate per established disease management protocols from CDFW. Strict biosecurity practices will be in place at all times.

Daily or alternate day passive tank transfers of pre-settled larvae will occur, in addition to partial water changes on a daily basis after larval settlement. This will allow for control of bacterial infestations and maintenance of necessary beneficial microbial communities on early and late stage veligers and less frequently on settled juveniles. Oxytetracycline and Gentamycin may also be used in addition to these methods.

Stress Management: To control stress to the black abalone during induced spawning attempts, abalone will not be exposed to elevated hydrogen peroxide for longer than the published guidelines (2.5 - 3 hours of exposure). All treated abalone will be washed in fresh seawater to remove residual levels to permit spawning. Thermal shock techniques, if needed, will be closely monitored and controlled. Suggested thermal treatments are short in duration (0.5 h) and will increase water temperatures by 3-4°C, but still stay within the tolerance range for this species (7-24°C).

Frequency of Spawning: Abalone will only be induced to spawn should the gonad conditioning criteria be met. It may require longer periods of time to condition these animals than previously reported. No more than four attempts to induce spawning will occur within a one year period. The number of individuals used will depend on the sex ratio of the captive animals. Buffered seawater containing hydrogen peroxide is the industry standard for induced spawning; however, the thermal shock method may be used if the former method fails to trigger spawning. Released eggs, swimming trochophores, and developing veligers will be subsampled to assess initial numbers of each stage. Settled veligers will also be enumerated microscopically. With this sequence, we will be able to determine the survival rate through all stages of development (% survival).

Disposition of Tissues: In the event of mortalities, tissues will be preserved for subsequent analysis and per the protocols developed for the White Abalone Recovery Program (see attached). These tissues will be maintained at SWFSC except for those sent to CDFW for post-mortem examination and to other labs (e.g., UW, NWFSC, CICESE) for analysis and/or research (e.g., genetic studies). Samples of larval and juvenile abalone will also be collected and preserved to document development. Tissue samples will also be taken for genetic purposes. These can be taken from fresh dead animals, or small clips of epipodial tissue from live animals. If using live animals, epipodial sampling is non-lethal and can be done in-situ (within the rearing tanks under water) or also when any other routine handling/sampling events occur. Epipodial sampling will occur to generate samples for genetic purposes and considered a one-time event per animal. Tissue samples may be maintained at the SWFSC as well as transferred to co-investigators at other facilities (UW, NWFSC, CDFW, CICESE) for analysis.

There are routine mortalities at every life stage in laboratory cultivation activities. Due to the high fecundity of black abalone, our spawning efforts may generate animals in excess of those required for the proposed research. Excess progeny produced under controlled conditions from abalone collected pre-listed ESA status, could be distributed to other institutions interested in life history aspects as well as support future outplanting activities. In the future, we may request to add additional facilities to this permit to conduct research activities using live black abalone at those facilities.

Lethal Take: Not Applicable

Anticipated Effects on Animals: Captive spawning: mild stress, no lethal effects. Growout activities of adults and spat: mild stress, mortality similar to commercial hatcheries, possible risk from Withering Syndrome or pumped seawater failure.

Measures to Minimize Effects: A total of 10 black abalone were transferred from SSC PACIFIC to SWFSC on December 19, 2014 (see attached Chain of Custody and transfer approval letter). Animals were placed within a flowing seawater and aerated quarantine system. There have been two mortalities since the animals were transferred. Fecal samples tested positive for WS on February 20, 2015. Animals will be treated with Oxytetracycline dihydrate per established disease management protocols from CDFW. Strict biosecurity practices will be in place at all times. All methods for moving abalone brood stock follow the "Abalone Risk Management Guidance Document," (NMFS, Draft 2/4/2011) and Appendix A (Broodstock collection and holding protocol) in the White Abalone Recovery Plan (NMFS 2008).

Resources Needed to Accomplish Objectives: The SWFSC has successfully maintained wild and cultured pink abalone broodstock since 2006, and more recently with green and red abalone. The SWFSC Aquarium facility is an established 80-100 gpm seawater flow-through facility located in La Jolla, CA, with ten different temperature mixing stations. Temperature is controlled to each mixing station by a centralized computer and also remotely monitored by two systems. Temperatures can be manipulated from 5 to 30°C at each of the mixing stations. All abalone are fed with fresh macroalgae two times a week and the facility is staffed or checked 7 days per week. Seawater flow and temperature monitoring systems with automated alarm notification capabilities are installed.

Disposition of Tissues: In the event of mortalities, tissues will be preserved for subsequent analyses. These tissues will be maintained at the SWFSC except for those sent to CDFW for post-mortem examination and to other labs (including the UW, NWFSC, and CICESE) for analysis and/or research (e.g., genetic studies). Larval and juvenile abalone will be collected and preserved in order to document development. These can be taken from fresh dead animals, or small clips of epipodial tissue from live animals. If using live animals, epipodial sampling is non-lethal and can be done in-situ (within the rearing tanks under water) or also when any other routine handling/sampling events occur. Epipodial sampling will occur to generate samples for genetic purposes and considered a one-time event per animal. Tissue samples may be maintained at the SWFSC as well as transferred to co-investigators at other facilities (UW, NWFSC, CDFW, or CICESE) for analysis.

Public Availability of Product/Publications: All information obtained to enhance broodstock conditioning, spawning techniques and larval and juvenile rearing will be made available to all interested parties. In addition we plan to create a protocol development report and publish these methods in a peer-reviewed journal:

"Methods for induced spawning in the black abalone, *Haliotis cracherodii*," (protocol development report).

"Hatchery methods for conditioning and induced spawning in the black abalone, *Haliotis cracherodii*," peer-reviewed publication to be sent to the Journal of Shellfish Research.

Federal Information

Federal Agency	Type	Authorization Number and Title	Date Signed	Expiration Date	Listing Units/Stocks Covered	Comments
National Marine Fisheries Service (NMFS)	Other	n/a			N/A	This research is entirely supported by federal personnel and funds. See attached Letter of Permission.

Location/Take Information

Location

Study Number 8 Research Area: Pacific Ocean State: CA

Location Description: Abalone are currently being maintained in a tank system at SWFSC

Take Information

Line	Ver	Species	Listing Unit/Stock	Production /Origin	Life Stage	Sex	Expected Take	Indirect Mort	Take Action	Observe /Collect Method	Procedure	Transport Record	Begin Date	End Date
1		Abalone, Black	California (NMFS Endangered)	Wild	Adult/Juvenile	Male and Female	24	24	Captive animals (research, enhancement, public display)	Captive	Captive, breed; Captive, lab experiments; Captive, maintain; Mark, other (e.g., neoprene patch); Measure; Mortality; Observation, monitoring; Tissue Sample Epipodial; Transfer/transport, live	1;2	8/12/2016	12/31/2020
Details: 24 black abalone = 8 from SSC Pacific (transferred 12/19/2014; see corresponding Chain of Custody) and 16 individuals from other captive facilities. Though unlikely, all the wild animals could die due to natural mortality or unusual mortality events.														
3		Abalone, Black	California (NMFS Endangered)	Captive	All	Male and Female	99999999	99999999	Captive animals (research, enhancement, public display)	Captive	Captive, breed; Captive, lab experiments; Captive, maintain; Mark, other (e.g., neoprene patch); Measure; Mortality; Observation, monitoring; Tissue Sample Epipodial; Transfer/transport, live	1;2	8/12/2016	12/31/2020

Details: We request to maintain and conduct research with all captive-bred progeny produced, or received from other facilities, under this permit. Though unlikely, all the captive-bred animals could die due to natural mortality or unusual mortality events.													
4	Abalone, Black	California (NMFS Endangered)	Wild	Adult/Juvenile	Male and Female	130	130	Captive animals (research, enhancement, public display)	Other	Captive, breed; Captive, lab experiments; Captive, maintain; Mark, other (e.g., neoprene patch); Measure; Mortality; Observation, monitoring; Tissue Sample Epipodial; Transfer/transport, live	1;2	8/12/2016	12/31/2020
Details: Up to 130 black abalone recovered from law enforcement actions, , emergency response activities, or Federal projects involving live black abalone. Though unlikely, all the wild animals could die from natural mortality or unusual mortality events.													

Transport Information

1. Mode(s) of Transportation: vehicular transport
Transportation Company: N/A
Maximum amount of time between capture and arrival: Variable
Container Description: Methods vary by life stage. Adults/juveniles: Place in coolers in moist towels. Embryos/larvae/small juveniles: Place in seawater-filled containers; place in a cooler. Cryopreserved gametes: Use cryopreservation containers.
Special Care: Temperature will be monitored and maintained with ice packs or other means to keep the animals cool and within their temperature range. Cryopreserved gametes will be kept at appropriate temperatures to prevent thawing
Accompanying Personnel Qualifications: All abalone will be quarantined until being tested for WS. Those that test positive for WS will be treated with Oxytetracycline dihydrate per established disease management protocols.
Facility Title: La Jolla Laboratory
Facility Affiliation/Organization: NMFS Southwest Fisheries Science Center
Address: 8901 La Jolla Shores Drive
La Jolla, CA 92037 UNITED STATES
Phone Number: (858)546-7000
Containment Method: Adults, juveniles: house in separate holding systems. Embryos/larvae: Rear using established methods and optimal conditions developed by research (see above). Cryopreserved gametes: Maintain at appropriate temperatures, for use in spawning events.
Final Disposition: Maintained at the SWFSC Aquarium Culture Facility.

2. Mode(s) of Transportation: Vehicular transport
Transportation Company: N/A

Maximum amount of time between capture and arrival:	Variable
Container Description:	Methods vary by life stage. Adults/juveniles: Place in coolers in moist towels. Embryos/larvae/small juveniles: Place in seawater-filled containers; place in a cooler. Cryopreserved gametes: Use cryopreservation containers.
Special Care:	Temperature will be monitored and maintained with ice packs or other means to keep the animals cool and within their temperature range. Cryopreserved gametes will be kept at appropriate temperatures to prevent thawing.
Accompanying Personnel Qualifications:	All abalone will be quarantined until being tested for WS. Those that test positive for WS will be treated with Oxytetracycline dihydrate per established disease management protocols.
Facility Title:	Center for Scientific Research and Higher Education of Ensenada, Baja California (CICESE)
Facility Affiliation/Organization:	CICESE Department of Aquaculture
Address:	Carretera Ensenada-Tijuana No. 3918, Fracc. Playitas 22860 Ensenada, MEXICO
Phone Number:	+52-175-0500 ext.
Containment Method:	Adults, juveniles: house in separate holding systems. Embryos/larvae: Rear using established methods and optimal conditions developed by research (see above). Cryopreserved gametes: Maintain at appropriate temperatures, for use in spawning events.
Final Disposition:	Maintained at the CICESE Department of Aquaculture Facility.

NEPA Checklist

1) If your activities will involve equipment (e.g., scientific instruments) or techniques that are new, untested, or otherwise have unknown or uncertain impacts on the biological or physical environment, please discuss the degree to which they are likely to be adopted by others for similar activities or applied more broadly.

Routine daily husbandry duties for captive abalone include feeding, cleaning tanks, system maintenance and observing and recording data at the SWFSC. Abalone are fed fresh kelp and other macroalgae that are collected as drift macroalgae on the shores of surrounding beaches in San Diego County in accordance with the SWFSC's Scientific Collection Permit (CDFW SC-12372). Because drift macroalgae are washed ashore and ultimately disposed of by city workers, no negative environmental impacts are anticipated. These activities are considered standard abalone industry practices; they are not considered new or untested.

2) If your activities involve collecting, handling, or transporting potentially infectious agents or pathogens (e.g., biological specimens such as live animals or blood), or using or transporting hazardous substances (e.g., toxic chemicals), provide a description of the protocols you will use to ensure public health and human safety are not adversely affected, such as by spread of zoonotic diseases or contamination of food or water supplies.

There is no known transmission of pathogens between abalone and humans. The proposed activities do not involve agents that threaten human safety. When abalone are treated for disease, induced to spawn, and reared through larval stages, antibiotics, and chemicals such as hydrogen peroxide and GABA may be used. These are either disposed of through San Diego city sanitary sewer lines or collected and disposed of using a commercial hazardous material disposal service depending on relevant discharge criteria. All sewer and seawater discharge is routinely monitored to ensure environmental compliance.

3) Describe the physical characteristics of your project location, including whether you will be working in or near unique geographic areas such as state or National Marine Sanctuaries,

Marine Protected Areas, Parks or Wilderness Areas, Wildlife Refuges, Wild and Scenic Rivers, designated Critical Habitat for endangered or threatened species, Essential Fish Habitat, etc. Discuss how your activities could impact the physical environment, such as by direct alteration of substrate during use of bottom trawls, setting nets, anchoring vessels or buoys, erecting blinds or other structures, or ingress and egress of researchers, and measures you will take to minimize these impacts.

The SWFSC Aquarium is an established 80-100 gpm seawater flow-through facility located in La Jolla, CA immediately adjacent to the Matlahuayl State Marine Reserve and San Diego – Scripps Coastal State Marine Conservation Area. Unfiltered seawater is pumped from the Scripps Institution of Oceanography pier up to the main laboratory building, where it is filtered through a series of sand filters, cartridge filters, UV sterilization units, ozone sterilization, and activated carbon filtration. The SIO seawater system infrastructure has been operating in its current state since 1988 and no changes to this infrastructure are required for the proposed research. Outgoing water is either discharged through sanitary sewer lines or the Scripps Institution of Oceanography's seawater return system. The seawater return is routinely monitored to ensure environmental compliance for discharge into this area. No other structures will be built near our facility and no environmental impact to the surrounding environment is anticipated.

4) Briefly describe important scientific, cultural, or historic resources (e.g., archeological resources, animals used for subsistence, sites listed in or eligible for listing in the National Register of Historic Places) in your project area and discuss measures you will take to ensure your work does not cause loss or destruction of such resources. If your activity will target marine mammals in Alaska or Washington, discuss measures you will take to ensure your project does not adversely affect the availability (e.g., distribution, abundance) or suitability (e.g., food safety) of these animals for subsistence uses.

This work will not affect entities listed in or eligible for listing in the National Register of Historic Places, or cause loss or destruction of scientific, cultural, or historic resources. Our activity in La Jolla, CA will not affect marine mammals or food safety of resident marine life in the area.

5) Discuss whether your project involves activities known or suspected of introducing or spreading invasive species, intentionally or not, (e.g., transporting animals or tissues, discharging ballast water, use of equipment at multiple sites). Describe measures you would take to prevent the possible introduction or spread of non-indigenous or invasive species, including plants, animals, microbes, or other biological agents.

Epiphytes could be attached to the black abalone that are also endemic to southern California waters. No invasive species problems associated with this project are envisioned as the sabellid worms have not been found at either SSC PACIFIC or at SWFSC. Both facilities have been certified sabellid-free from CDFW. Black abalone tested positive for WS on February 20, 2015. They will be treated with Oxytetracycline dihydrate per established disease management protocols from CDFW.

Project Contacts

Responsible Party: Francisco Werner

Primary Contact: John Hyde

Principal Investigator: John Hyde

Other Personnel:

Name	Role(s)
Patrick Appel	Co-Investigator
Matthew Craig	Co-Investigator
Carolyn Friedman	Co-Investigator
Fabiola Lafarga De la Cruz	Co-Investigator

Jim Moore	Co-Investigator
Catherine Purcell	Co-Investigator
Piper Schwenke	Co-Investigator

Attachments

Certification of Identity - P19571T1119571_SWFSCLaJolla_SignedAuthentication_Sec109_30_2015.pdf (Added Sep 30, 2015)
Contact - Carolyn Friedman C17392T5BIOGRAPHY Friedman 2013.doc (Added Jan 6, 2014)
Contact - Fabiola Lafarga De la Cruz C18844T5CV_Fabiola_Feb2015-1.pdf (Added Jul 23, 2015)
Contact - John Hyde C18846T5Relavent Experience Hyde.pdf (Added Jul 23, 2015)
Contact - John Hyde C18959T5JRHydeCV.doc (Added Sep 22, 2015)
Contact - Piper Schwenke C13040T51346_PiperCV2009.doc (Added May 4, 2009)
Project Description - P19571T1Aquilino_2015_05_WhiteAbaloneSpawningandCulturingGuide.pdf (Added Sep 22, 2015)
References - P19571T1215DEC2014_SWFSC Abalone_JJ.pdf (Added Jul 10, 2015)
References - P19571T12COC_Black Abalone 12-19-2014.pdf (Added Jul 10, 2015)

Status

Application Status:	Application Complete
Date Submitted:	September 30, 2015
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FR Notice of Receipt Published:	October 14, 2015 Number: 2015-25985
Comment Period Closed:	November 13, 2015 Comments Received: No Comments Addressed: No
Last Date Archived:	August 18, 2016

- ESA Section 10(a)(1)(A) permit (Pacific fish/invertebrate research)

Current Status: Issued Status Date: August 12, 2016

Section 7 Consultation: Formal Consultation

NEPA Analysis: Categorical Exclusion

Date Cleared by General Counsel: June 29, 2016

Expire Date: December 31, 2020

Analyst Information:

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Reports

Reports Required

Nbr	Report Type	Report Period		Date Due	Status	Date Received
		Start Date	End Date			
1	Annual-Year End	08/12/2016	12/31/2016	01/31/2017	N/A	
2	Annual-Year End	01/01/2017	12/31/2017	01/31/2018	N/A	
3	Annual-Year End	01/01/2018	12/31/2018	01/31/2019	N/A	
4	Annual-Year End	01/01/2019	12/31/2019	01/31/2020	N/A	
5	Annual-Year End	01/01/2020	12/31/2020	01/31/2021	N/A	